

Optical coherence tomography angiography changes in the three parafoveal retinal plexuses in response to hyperoxia

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Conflict of Interest

Oregon Health & Science University (OHSU), Yali Jia and David Huang have a significant financial interest in Optovue, Inc., a company that may have a commercial interest in the results of this research and technology. These potential conflicts of interest have been reviewed and managed by OHSU. David Huang receives patent royalties from Carl Zeiss Meditec, Inc. Other authors do not have financial interest in the subject of this article.

Running head

OCTA changes in parafoveal retinal plexuses after hyperoxia

Abstract

Purpose: Use projection-resolved optical coherence tomography angiography (PR-OCTA) to investigate the autoregulatory response in the three parafoveal retinal plexuses under hyperoxia.

Design: Prospective cohort study.

Subjects: Nine eyes from nine healthy participants.

Methods: One eye from each participant was scanned using a commercial spectral-domain OCT system. Two repeated macular scans (3 x 3 mm²) were acquired at baseline and during oxygen breathing. Split-spectrum amplitude-decorrelation algorithm (SSADA) was used to detect blood flow. The PR algorithm was used to suppress projection artifacts and resolve blood flow in three distinct parafoveal plexuses. The Wilcoxon signed-rank test was used to compare between baseline and hyperoxic parameters. Coefficient of variation (CV), intra-class correlation coefficient (ICC), and pooled standard deviation (SD) were used to assess the reliability of OCTA measurements.

Main Outcome Measures: Flow index and vessel density were calculated from the *en face* angiograms of each of the three plexuses, as well as from the all-plexus inner retinal slab.

Results: Hyperoxia induced significant reduction in the flow index (-11%) and vessel density (-7.8%) of only the deep capillary plexus (DCP, $p < 0.001$) and in the flow index of the all-plexus slab ($p = 0.015$). The flow index also decreased in the intermediate capillary plexus (ICP) and the superficial vascular complex (SVC), but these changes were small and not statistically significant. The PR-OCTA showed good within-session baseline repeatability (CV, 0.8%-5.2%, ICC, 0.93-0.98) in all parameters. Relatively large between-day response reproducibility was observed (pooled SD, 1.7%-9.4%).

Conclusions: Projection-resolved OCTA was able to show that the retinal autoregulatory response to hyperoxia affects only the DCP, but not the ICP or SVC.

The retina is one of the most metabolically active tissues of the human body.^{1, 2} A healthy retina possesses an intrinsic autoregulatory mechanism that modifies blood flow in response to different physiologic conditions in order to maintain homeostasis.^{3, 4} Impairments in the autoregulatory response have been associated with the pathogenesis of several vision-threatening diseases such as diabetic retinopathy (DR) and age-related macular degeneration (AMD).⁵⁻¹⁰ Physiologic variations in oxygen delivery to retina was found to induce multiple hemodynamic changes.^{11, 12} The ability to characterize this response can be useful to investigate the retinal vascular physiology as well as pathophysiology of those diseases.

Several noninvasive imaging modalities such as blue field entopic phenomenon,^{13, 14} scanning laser Doppler techniques^{15, 16} and Doppler optical coherence tomography (OCT)¹⁷⁻¹⁹ have been used to investigate autoregulation. These studies reported decrease in retinal blood velocity, vessel diameter and blood flow during increased tissue oxygenation (hyperoxia). More recently, OCT angiography (OCTA) has also been used to characterize a similar response in peripapillary inner retinal flow index and vessel density during hyperoxia.^{20, 21} Previous investigations described the hyperoxic response in the combined retina as a single vascular unit. However, histological studies in primates and human cadavers divided retinal circulation around macula into three distinct vascular plexuses.²²⁻²⁴ Bearing in mind the findings of oxygen distribution and consumption in different retinal layers,²⁵⁻²⁸ we predict that different retinal vascular layers might be reacting differently to changes in systemic oxygenation. Still, traditional imaging modalities lacked the capability to resolve the flow in these three plexuses separately, and hence we had limited understanding of how they react separately to oxygen.

We have recently developed an algorithm called the projection-resolved²⁹ OCT angiography (PR-OCTA) which allows for the visualization and segmentation of the three

distinct parafoveal vascular plexuses of the inner retina: superficial vascular complex (SVC), intermediate capillary plexus (ICP) and deep capillary plexus (DCP).³⁰ In this study, we aim to characterize the change in flow index and vessel density of the parafoveal retinal plexuses in response to hyperoxia using PR-OCTA.

Methods

Study population

The study was conducted at Casey Eye Institute and the protocol was approved by the Institution Review Board of Oregon Health and Science University. We adhered to the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act in the treatment of human subjects. The nature of the study was explained to each participant and an informed written consent was obtained. Healthy volunteers were recruited to participate in this study. Volunteers were excluded if they had one of the following: ocular disease, inability to maintain stable fixation for scanning, visual acuity worse than 20/40, refractive errors more than -6.00 or +2.00 diopters, significant media opacity, or history of major ocular surgery, as well as systemic diseases that can affect microcirculation (such as diabetes and hypertension).

OCT Angiography

The experiment was conducted using the commercially available spectral-domain OCT system (RTVue-XR Avanti, Optovue, Inc., Fremont, CA). The system has an A-scan repetition rate of 70 kHz with a center wavelength of 840 nm and a bandwidth of 45 nm. The axial and transverse resolutions in tissue are 5 μ m and 22 μ m, respectively.

Volumetric scans, centered at the macula, were 3×3 mm with a depth of 1.6 mm. B-scans in the fast transverse scanning direction were comprised of 304 A-scans. Two consecutive

B-scans were captured at each location before proceeding to the next location. A total of 304 locations in the slow transverse direction were sampled. This formed a 3D data cube consisting of $304 \times 304 \times 512$ pixels. The 608 B-scans in each data cube were acquired in 2.9 seconds. Two volumetric raster scans, one x-fast scan and one y-fast scan, were obtained and registered. This scan set was repeated a second time during each scan session. A scan session was included if at least one scan set was free of motion artifacts.

Both the blood flow and structural information were obtained congruently using the AngioVue software, a commercial version of the split-spectrum amplitude-decorrelation angiography (SSADA) algorithm. A detailed description of the SSADA method can be found in our previous publications.^{21, 31} In short, the SSADA algorithm splits the spectrum into 11 sub-spectra and calculates the signal amplitude-decorrelation between the two consecutive B-scans captured at each location. This method enhances the signal-to-noise ratio of flow detection.³² The decorrelation signal, representing here the blood flow signal for OCT angiography, is a function of light scattering due to the displacement of red blood cells over time. The subspectra used in SSADA have $\frac{1}{4}$ of the full OCT bandwidth and supports an angiographic depth resolution of 20 μm , which is sufficient to resolve the retinal plexuses.

Data Acquisition

Experiments were performed in a quiet and dimly lighted room. During the first scan session, participants were asked to sit and breathe normally for 10 minutes to establish a baseline condition. Arterial blood pressure and pulse were taken and recorded. Participants were then asked to fixate on an internal fixation target. The baseline breathing condition was maintained while the operator used the real-time video image on the software interface to center at macula and acquire the 2 scan sets (constituting 1 scan session). The second scan session immediately

followed, during which participants were fitted with a simple face mask (OxyMask, Southmedic, Barrie, Ontario, Canada) and given supplemental oxygen for 10 minutes at a flow rate of 15 liters per minute. This rate delivered about 60-90% oxygen in the inspired oxygen (FiO_2), creating a systemic hyperoxic condition. These FiO_2 values were taken from mask's manufacturer's manual. Generally, the calculation of FiO_2 is based on the assumption that at a constant supplemental O_2 concentration and flow rate, FiO_2 depends on the individual's peak inspiratory flow rate, normally ranging around 20-30 L/min. At the end of the the 10 minutes, hyperoxia was maintained while blood pressure and heart rate were recorded and the participant's eye was scanned. In order to assess between-day reproducibility of the baseline measurement and hyperoxic response, the protocol was repeated on a separate day.

Data Processing

Participants would be excluded if both scan sets from a single scan session were of poor image quality (marked motion artifacts, unfocused, off-center, or low signal strength). The participant was included if at least one scan set from each scan session passed the quality control. Using the directional graph search technique, structural OCT images were automatically segmented at the inner limiting membrane (ILM), nerve fiber layer (NFL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor inner/outer segment (IS/OS) junction, retinal pigment epithelium (RPE), and Bruch's membrane (BM). Boundary lines defined on structural images were applied to corresponding angiography frames. Details about these methods are described in previous publications.³⁰

A detailed description of the projection-resolved algorithm can also be found in previous publications.²⁹ In short, PR-OCTA uses a linear model to relate the decorrelation value of projected flow with its log amplitude OCT signal and the overlying *in situ* flow. This assumes

that both *in situ* and projected decorrelation values are affected by the reflectance amplitude. Therefore, the projected flow signal is lower than the *in situ* flow signal in a linear fashion. This allowed the visualization of the 3 distinct retinal vascular plexuses in the inner parafoveal area without significant flow projection artifact.

En face angiograms were constructed by maximum flow projection within relevant slabs. The inner retinal slab was defined between the ILM and OPL (Figure 1). The inner retina was subdivided into 3 slabs corresponding to the 3 retinal plexuses commonly described in histological studies and visualized on composite OCT B-scan of the parafoveal region. SVC slab contains flow projection in the inner 80% of ganglion cell complex (GCC: layers between NFL and IPL), ICP is located in the outer 20% of GCC and inner 50% of INL, and DCP shows the flow in the outer 50% of INL and all of OPL.

Flow index and vessel density were automatically calculated from decorrelation values of pixels contained in the annulus region of the *en face* retinal angiograms (Figure 1). The annulus region was manually centered at the foveal avascular zone (FAZ) with the internal and external diameters set at 0.6 and 2.5 mm, respectively. Flow index is the average pixel decorrelation value and contains information on both vessel area and blood flow velocity. However, it cannot be considered true volumetric blood flow due to the saturation of decorrelation values in large vessels with fast velocities. Vessel density is the percent area of pixels with decorrelation values above a threshold defined as two standard deviation about noise at the FAZ.

Statistical Analysis

Statistical analysis was performed using Microsoft Excel 2013 (Microsoft office, Microsoft Corporation, Redmond, WA) and SPSS v. 24.0 (IBM Corporation, Armonk, NY). A paired *t*-test was used to compare heart rate (HR) and mean arterial pressure (MAP) between baseline and

hyperoxia. The 2 consecutive scan sets (if both were included) were averaged for each scan session. The parafoveal inner retinal flow index and vessel density in the three vascular plexuses and inner retina were presented as mean and standard deviation (SD) of all baseline and hyperoxia scan sets from all scan sessions. The Wilcoxon signed-rank test was used to compare baseline and hyperoxic flow index and vessel density. Change in these measurements during hyperoxia was calculated as the average change and standard deviation of all participants during both testing days. Coefficient of variation (CV) and intra-class correlation coefficient (ICC) with 95% confidence interval (CI) were used to measure within-session baseline repeatability. Between-day hyperoxic response reproducibility was measured using pooled SD after averaging the measurements of the two scans in each scan session.

Results

Nine participants (3 females and 6 males; average age, 27.2 ± 5.2 years) were enrolled and scanned on day 1. Eight participants were scanned on both days. A total of 67 scans were acquired, seven were excluded because of motion artifact. No participants or scan sessions were excluded from the study. Average HR for baseline and hyperoxia were 73.5 ± 3.1 and 72.5 ± 7.46 beat/min, respectively. Average MAP was 84.5 ± 8.9 and 81.0 ± 7.5 mmHg for baseline and hyperoxia, respectively. There was no significant difference between the two conditions in HR or MAP (*t*-test, $p > 0.05$).

Projection-resolved OCTA showed good within-session repeatability in baseline flow index and vessel density measurements in all retinal vascular plexuses (Table 1). Generally, the flow index has larger measurement variability than vessel density as measured by the CV and ICC. For most plexuses, the population variation of baseline measurements tended to be smaller for the vessel density compared to the flow index (Table 1). Both flow index and vessel density

declined after breathing oxygen in most plexuses. This hyperoxic response was statistically significant ($p < 0.001$) in the DCP, but not in the SVC or ICP. The hyperoxic response was larger for the flow index relative to the vessel density. However, the population variability of the hyperoxic response was also proportionally larger for the flow index. The decrease in flow signal in response to hyperoxia could be directly appreciated on the *en face* PR-OCT angiograms of the DCP (Figure 2).

A consistent reduction of flow index and vessel density in deep plexus was observed in almost all subjects on both days (Figure 3). However, the magnitude of response can change between days, as evidenced by the relatively large between-day response reproducibility (Table 2). For both vessel density and flow index, the variability in the hyperoxic response was approximately twice that of the repeatability of the baseline measurements. Thus the response variability is partially but not entirely explained by measurement noise.

Discussion

Autoregulatory mechanisms ensure a relatively constant supply of oxygen through a wide range of variation in blood oxygen content. Hyperoxia reduces retinal blood flow as measured by Doppler OCT,¹⁹ blue field entopic technique,^{13, 14} laser Doppler flowmetry,¹⁵ and laser Doppler velocimetry.¹⁶ In our previous OCTA investigation, we also found significant reduction in the flow index and vessel density in the inner retinal circulation around the optic disc.²⁰

The retinal vascular autoregulation is actually more complicated than a uniform homeostatic response due to interactions with the choroid. The choroidal circulation is thought to provide relatively constant flow,³³⁻³⁶ while the retinal circulation varies in response to changes in partial pressure of oxygen (pO_2).³⁷ In recent animal hypoxia experiments by Yi *et al.*, retinal blood flow increased so much that total oxygen extraction actually increased.²⁷ Assuming that

inner retinal oxygen consumption is constant during hypoxia,³⁸ they hypothesized that the increase in retinal oxygen extraction must have been caused by increased need to supply the outer retina to compensate for the drop in oxygen flux from the choroidal circulation.²⁷ This hypothesis is based on the fact that the avascular outer retina is supplied by both the retinal and choroidal circulations, which had been established by microelectrode oxygen tension measurements in animals.^{25, 39, 40}

Based on the results by Yi et al.,²⁷ we further hypothesize that the autoregulatory vascular response to changes in oxygen concentration would be greatest in the DCP, which supplies the outer retina. Because the choroidal flow does not respond to pO_2 , the fraction of outer retina oxygenated by the choroid would change with its oxygen content, with the DCP flow overcompensating to cover the need. This hypothesis can now be directly tested using the PR-OCTA algorithm²⁹ that could visualize and measure the DCP separate from the ICP and SVC. We expected to find that the DCP would have a greater reduction in flow index and vessel density during hyperoxia. The SVC and ICP blood flow should also be reduced, but to a much small extent, as they only need to maintain inner retinal oxygen flux.

Our expectations were confirmed by the experimental findings shown in this paper. The difference in hyperoxia-evoked autoregulation of blood flow between the three retinal vascular layers were striking. Hyperoxia induced significant reductions in the flow index and vessel density of in the DCP. In contrast, the reduction of flow index and vessel density in the SVC and ICP were too small to be statistically significant. The explanation is that during oxygen breathing, the oxygen tension in the avascular outer retina increases^{26, 36, 41} due to greater supply from the choroidal circulation, which maintains constant flow despite greater pO_2 .³³⁻³⁵ Thus to maintain steady oxygen flux in the outer retina, the DCP must compensate with greater

vasoconstriction and reduced flow. This maintains the oxygen tension in the outer retina at a low enough level to prevent potential damage from oxidative stress.^{42, 43} The reduction in DCP flow would in turn reduce oxygen flux to the mid-retina and thereby blunt the response of ICP and SVC to systemic hyperoxia.

The changes we measured in the DCP and all-plexus slabs (11% and 3.5%, respectively) were in the flow index. Flow index is calculated by averaging the decorrelation value in the parafoveal region. Decorrelation is not linearly related to blood velocity – laboratory measurements using the current OCTA system showed that the decorrelation-velocity curve begins to flatten around 2 mm/second and reaches saturation at approximately 6 mm/second.⁴⁴ Thus, the percentage reduction in flow index was expected to be smaller than the change in volumetric blood flow as measured by laser Doppler¹¹ or Doppler OCT techniques.¹⁹

The differential regulation of capillary plexuses has been previously reported in the retina and cerebral cortex. Kornfield and Newman observed that flicker stimulation in rats can produce much larger response in the retinal ICP than in the SCP and DCP.⁴⁵ Similarly, observations in cortical vascular layers in response to sensory stimulation indicated greater increases in blood flow in the middle capillary layers.⁴⁶ In contrast to arterioles and venules, capillaries lack smooth muscles in their walls. Thus, the mechanism by which capillaries can actively regulate their blood flow independently from the more superficial arteriolar layers is still not fully understood. Studies on somatosensory cortex give evidence that pericytes might be responsible for blood flow regulation in capillaries.⁴⁷ Contraction of pericytes in response to hyperoxia can produce constriction of deep capillaries and consequently increasing vessel wall resistance and decreasing blood flow. Moreover, the regulation of DCP blood flow might be happening at the level of pre-capillary sphincter or the penetrating arterioles connecting the ICP to the DCP.⁴⁸

Prior to the development of PR-OCTA, it would have been difficult to noninvasively study the retinal plexuses. Classically, fluorescein angiography (FA) and indocyanine green angiography (ICGA) have been used to image the ocular circulation.⁴⁹⁻⁵¹ However, these two-dimensional imaging modalities cannot separate retinal and choroidal circulations, let alone resolve retinal plexuses.^{52, 53} With the introduction of OCTA technology, three-dimensional angiograms of the retinal and choroidal circulations could be separated.^{54, 55} However, the retinal plexuses still could not be cleanly separated due to the flow projection artifact that projects flow signal from the superficial retinal vessels onto the deeper structures.⁵⁶ The projection artifacts would cause flow from the SVC and ICP to be projected onto the DCP,^{55, 57} making it impossible to accurately measure flow and vessel density in the DCP.

The projection-resolved algorithm distinguishes between the true and false positive flow signals by comparing the intensity-normalized decorrelation value in each voxel to all of the more superficial voxels in the same A-scan line, taking into account that projected signal is usually weaker than the true *in situ* source flow signal.²⁹ This method effectively resolved the projection artifacts on both *en face* and cross-sectional angiograms and improved the axial resolution of OCTA, allowing for better visualization of the three parafoveal vascular plexuses with proper quantification of vessel density and flow index. PR-OCTA showed very good within-session repeatability for flow index and vessel density measurements for the inner retina as a whole. However, the repeatability for single plexuses, and particularly for the DCP, was slightly worse.

We observed a relatively large between-day variation in the hyperoxic response that could not be entirely explained by measurement error as reflected in within-session repeatability. The excess variability could be due to variations in the internal physiological state and

environmental factors. In order to reliably measure individual hyperoxic response magnitude, it may be necessary to average response from several stimulus cycles.

Alterations to hemodynamics of retinal circulation and its normal physiologic responses are suggested to be involved in pathophysiology of multiple retinal diseases such as age-related macular degeneration, diabetic retinopathy, and branch retinal vein occlusion.^{5-7, 37, 58, 59} Deep understanding of these associations can serve as a basis for future efforts of developing more effective and efficient diagnostic and therapeutic strategies. PR-OCTA has the potential to be useful in investigating autoregulation dysfunction in retinal diseases.

The main limitation in this pilot study is the small sample size. Additionally, our results were based on observations from a single time-point after systemic hyperoxia had already been established. However, the hypothesis and findings at this stage are useful for designing larger studies to confirm the results and provide dynamic analysis of hyperoxic response in retinal microcirculation.

In summary, PR-OCTA demonstrated the capability of visualizing and measuring individual retinal plexuses, allowing for further physiological investigations. We reported a significant decrease of flow index in the retina in response to systemic hyperoxia. This response was found to be specifically caused by the change in the DCP but not the ICP or SVC; giving a new evidence of the interplay between choroidal and retinal circulation in oxygenating the avascular outer retina.

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Figure Legends

Figure 1. Projection-resolved optical coherence tomography angiography (PR-OCTA) images from a baseline scan session. (A-C) *En face* maximum flow projection angiograms for the three parafoveal retinal plexuses: superficial vascular complex (SVC), intermediate capillary plexus (ICP), deep capillary plexus (DCP). Flow index and vessel density were calculated in the annulus region between the two green circles. (D) Cross-sectional PR-OCTA with gray-scale reflectance signal and color-coded flow signal. The anatomic boundaries (dotted lines) demonstrate the results of automated computer segmentation of inner retinal layers and are used to derive the slab boundaries of the retinal plexuses. The relevant retinal layers were inner limiting membrane (ILM), ganglion cell complex [GCC: including nerve fiber layer (NFL), ganglion cell layer (GCL), and inner plexiform layer (IPL)], inner nuclear layer (INL), and the outer plexiform layer (OPL).

Figure 2. *En face* angiograms of superficial, intermediate and deep parafoveal vascular plexuses of the same participant during baseline scan session (A-C), and after hyperoxia (D-F). Note the difference in flow signal (color scale) and vessel density between baseline and hyperoxia. The decrease is visually apparent in the deep capillary plexus (DCP), but not in the superficial vascular complex (SVC) or intermediate capillary plexus (ICP).

Figure 3. Deep vascular plexus hyperoxic response in flow index (left) and vessel density (right) for each participant on both days.